THE EFFECT OF DESICCATION ON CELL SHAPE IN THE LICHEN *PARMELIA SULCATA* TAYLOR

BY D. H. BROWN[1], S. RAPSCH[2], A. BECKETT[1] AND C. ASCASO[2]

[1]*Department of Botany, The University, Bristol BS8 1UG, UK and*
[2]*Instituto de Edafologia y Biologia Vegetal, C.S.I.C., Serrano 115 bis, Madrid 28006, Spain*

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SUMMARY

Comparisons have been made of the shape of algal cells in the lichen *Parmelia sulcata* which was subjected to controlled desiccation regimes inducing substantial water loss. The spherical appearance of algal cells obtained by conventional techniques for transmission electron microscopy (TEM) was shown by low temperature scanning electron microscopy (LTSEM) to be the consequence of rapid rehydration during fixation. Collapse of the walls of algal cells and fungal hyphae in the medulla and algal layer when desiccated were observed with LTSEM and shown to be reversible on rehydration. Desiccation-induced contraction of cortical fungal protoplasts was detected with LTSEM. Fixation with osmium vapour only before TEM demonstrated a similar contraction of algal protoplasts.

Key words: Lichen, desiccation, cell shape, contraction, LTSEM, *Parmelia sulcata*.

INTRODUCTION

Lichens lack active mechanisms for controlling gain and loss of water and, under normal environmental conditions, most lichens experience periods of desiccation. The consequences of desiccation treatments which are either extreme or ecologically justifiable have been studied by physiologists (see references in Bewley, 1979; Kershaw, 1985) and electron microscopists (see references in Ascaso, Brown & Rapsch, 1985, 1986). Ultrastructural studies have been confined to transmission electron microscopy (TEM) of intracellular changes and freeze-fracture studies of membrane surfaces. Such studies require tissues to be fixed in aqueous media, and this procedure may constitute a period of rehydration. No observations have been made of whether water loss causes changes in the cell wall or protoplast shape while the material is still dehydrated. We have investigated this using a variety of techniques.

MATERIALS AND METHODS

Parmelia sulcata Taylor was collected from bushes of *Salix* near Priddy, Mendip Hills, Somerset (ST 533524) and stored on moist filter paper for 48 h in a 12 h light (135 μmol m^{-2} s^{-1}), 20 °C/12 h dark, 14 °C regime. Samples were then transferred to small sealed Perspex boxes maintained at 100 % RH (water-saturated filter paper), 53 % RH (saturated magnesium nitrate solution) or 0 % RH (activated silica gel) for 72 h, unless otherwise stated. For conventional TEM, samples from 2 to 3 mm behind the lobe margin were fixed at 4 °C for 3 h in 3·125 % glutaraldehyde in 0·05 mol dm^{-3} sodium phosphate buffer, pH 7·1. Samples were washed for 12 h in phosphate buffer and postfixed in 1 % osmium

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tetroxide in phosphate buffer for 3 h. Material was dehydrated in graded ethanol solutions and embedded in Spurr's resin. Sections were stained with Reynolds' lead citrate and examined with a Philips EM 300 electron microscope.

For low temperature scanning electron microscopy (LTSEM), samples were taken from the same thallus position, quench-frozen in liquid nitrogen, cross-fractured in an EMscope SP2000 cryogenic preparation unit and either viewed directly using a Philips 501B SEM or etched while under observation on the microscope cold stage by raising the temperature from −170 to −69 °C and etching for 7·5 min. Samples were usually sputter-coated with gold before photography.

Some samples were fixed by exposure to osmium vapour only. These samples had been stored under the above light/dark regime at 0% RH for four months before being either rehydrated in an atmosphere of 100% RH for 48 h at room temperature or used while still dry. Samples were then exposed to osmium tetroxide vapour for 20 h in 4·5 ml stoppered tubes at room temperature, rinsed and dehydrated in 100% ethanol and embedded in Spurr's resin as above.

RESULTS AND DISCUSSION

Water content

It was impossible to obtain direct estimates of the water content of the samples used in these studies because thalli are unavailable for dry weight determinations after microscopical examination. Estimates based on water loss were also subject to variation due to the variability of the initial water content of the sample. Estimates from the samples used for conventional TEM and initial LTSEM showed weights after storage as a percentage of the initial fresh weight (imbibed and surface water removed by blotting) of 100% RH = 97·6 ± 2·7%, 53% RH = 61·7 ± 2·5% and 0% RH = 55·0 ± 1·4% compared to 54·3 ± 0·8% for oven-dried material.

Transmission electron microscopy

Studies using conventional techniques of fixation, sectioning and staining for TEM have shown that the algal cells (*Trebouxia*) in sections of the thallus of *P. sulcata* were roughly circular and bounded by a circular cell wall. This spherical form was most apparent when cell-to-cell contacts were minimal [Fig. 1(a), (b)]. Sectioned algal cells growing embedded in the base of the close-packed upper cortex of fungal hyphae still showed circular outlines in those parts not constrained by other cells [Fig. 1(c)]. No difference in the form of algal cell walls could be detected after storage at 100% RH [Fig. 1(a)], 53% RH or 0% RH [Fig. 1(b), (c)]. There was, however, a tendency for the protoplast to become non-circular in section and to pull away from the inner surface of the cell wall after storage at 0% RH. This was most obvious in algal cells embedded in the base of the upper cortex [Fig. 1(c)]. These results suggest that desiccation does not induce major changes in the cell-wall shape of *Trebouxia* cells.

When four month-stored samples for TEM were fixed in osmium tetroxide vapour only, preservation of intracellular structures was poor and cell walls were not detectable (Fig. 2). It was obvious that the protoplasts of samples stored at 0% RH were highly contracted and convoluted [Fig. 2(a)]. Osmium uptake was mostly confined to surface structures in dried material, whereas rehydrated material showed a more general osmium uptake and fixation. Rehydrated cells

showed a substantially more circular appearance in section, but some contraction was still apparent [Fig. 2(b)]. As the dark-staining bodies responsible for the crenulate outline of the dried sample [Fig. 2(a)] resemble the storage bodies immediately beneath the plasma membrane [Fig. 1(a)], these micrographs show the form of the contracted protoplast and not just the chloroplast shape.

The capacity to reform a circular protoplast on rehydration showed that dry storage for four months did not damage the permeability properties of the plasma membrane severely. Rehydration at 100 % RH is a slower and less complete process than immersion in liquid water (Kershaw, 1985) and hence full recovery from the highly contracted state at 0 % RH may not have occurred. Equally, minor contractions of the protoplast have been detected in conventionally fixed material stored at 100 % RH [Fig. 1(a)], where some water loss is known to occur from the fully hydrated condition (see above). When using osmium vapour fixation, samples were placed directly into 100 % ethanol before embedding in Spurr's resin. While such a desiccant treatment could have caused some protoplast contraction, it is unlikely to have accounted for the difference between the protoplast shapes in the dried and rehydrated samples.

Low temperature scanning electron microscopy

The use of LTSEM enables samples to be frozen, coated and observed rapidly without the use of liquid fixatives (Beckett & Read, 1986). Using this technique, it was apparent that algal and fungal cells in *P. sulcata* thalli stored at 100 % RH were fully inflated [Fig. 3(a)], whereas storage at 53 % or 0 % RH [Fig. 3(b)] caused marked cellular contraction. Compared to the smooth, inflated appearance at 100 % RH, algal cells became highly wrinkled and convoluted after storage at 0 % RH. At 53 % RH the algal cells were also obviously collapsed to varying degrees but not to the extremes shown at 0 % RH. When material stored at 0 % RH was rehydrated for 2 min in either deionized water [Fig. 4(a)] or glutaraldehyde in phosphate buffer [Fig. 4(b)], algal cells became reinflated.

Fungal hyphae in the medulla and algal layers were also contracted after storage at 0 % RH, as shown by longitudinal folds on the hyphal walls and by the greater hyphal diameter at the position of the septum between hyphal cells [Fig. 3(b)]. The close packing of fungal hyphae in the cortex reduced their ability to contract on desiccation. At 100 % RH, cortical hyphae contained inflated protoplasts which either remained as protrusions or, if pulled out during fracturing, left depressions in the fracture face [Fig. 3(a)]. As the protrusions remained after etching at −69 °C, they do not represent ice crystals embedded in empty cavities of dead fungal hyphae. TEM shows all cortical cells to contain protoplasts. Similar arguments apply to the fungal protoplasts present in medullary hyphae. Samples desiccated at either 53 % or 0 % RH lack such smooth protoplast surfaces, and all hyphae appear as empty tubes, probably due to shrinkage of the fungal protoplast [Fig. 3(b)].

Medullary hyphae were covered in conspicuous needle-shaped crystals [Fig. 4(a)]. These were present in samples at both 100 % and 0 % RH and did not alter on etching. They do not, therefore, represent ice crystals but are probably phenolic lichen depsides. The algal cells were almost devoid of such crystals. On rehydration in water or glutaraldehyde-phosphate buffer, no obvious ice crystals appeared, suggesting that rehydration of algal cells occurred via the walls of fungal hyphae and not through the intercellular spaces. This supports the suggestion that phenolic crystals in lichens may function as water-repellent materials, ensuring

adequate gaseous pathways within the thallus for carbon dioxide diffusion to the algal cells (Green, Snelgar & Wilkins, 1985).

In fully hydrated algal cells, fractured cells showed the protoplast to be fully inflated [Figs 3(a), 4(a), (b)]. After glutaraldehyde-phosphate buffer rehydration, the plasma membrane surface appeared to be somewhat pock-marked [Fig. 4(b)]. Ridges were seen on fractured plasma membranes of algal cells which were fully hydrated with water [Fig. 5(a)]. These ridges, which were 200 to 600 nm long [Fig. 5(a)], have the same dimensions as those found in freeze-fractured samples of other symbiotic *Trebouxia* cells (Peveling & Robenek, 1980; Ascaso *et al.*, 1985). Dehydrated algal cells failed to fracture, and hence it was not clear whether their protoplasts had shrunk away from the convoluted cell walls [Fig. 3(b)].

Numerous fungal/algal connections were detected by LTSEM. Because there was no clear evidence of penetration through the algal cell wall, these are of an appressorial or interparietal nature [Figs 4(a), (b) and 5(b)]. Substantial adhesion occurred between the fungal and algal cells, as shown by the small fragments of fungal hyphal wall remaining attached to algal cell walls. At these points, small projections may occur through the algal cell wall [Fig. 5(b)] which were seen as depressions in the internal surface of the fungal wall.

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REFERENCES

ASCASO, C., BROWN, D. H. & RAPSCH, S. (1985). Ultrastructural studies of desiccated lichens. In: *Lichen Physiology and Cell Biology* (Ed. by D. H. Brown), pp. 259–274. Plenum Press, New York.

ASCASO, C., BROWN, D. H. & RAPSCH, S. (1986). The ultrastructure of the phycobionts of desiccated and hydrated lichens. *Lichenologist*, **18**, 37–46.

BECKETT, A. & READ, N. D. (1986). Low-temperature scanning electron microscopy. In: *Ultrastructure Techniques for Microorganisms* (Ed. by H. Aldrich & W. Todd), pp. 45–86. Plenum Press, New York.

BEWLEY, J. D. (1979). Physiological aspects of desiccation tolerance. *Annual Review of Plant Physiology*, **30**, 195–238.

GREEN, T. G. A., SNELGAR, W. P. & WILKINS, A. L. (1985). Photosynthesis, water relations and thallus structure of Stictaceae lichens. In: *Lichen Physiology and Cell Biology* (Ed. by D. H. Brown), pp. 57–75. Plenum Press, New York.

KERSHAW, K. A. (1985). *Physiological Ecology of Lichens*. Cambridge University Press, Cambridge.

PEVELING, E. & ROBENEK, H. (1980). The plasmalemma structure in the phycobiont *Trebouxia* at different stages of humidity of a lichen thallus. *New Phytologist*, **84**, 371–374.

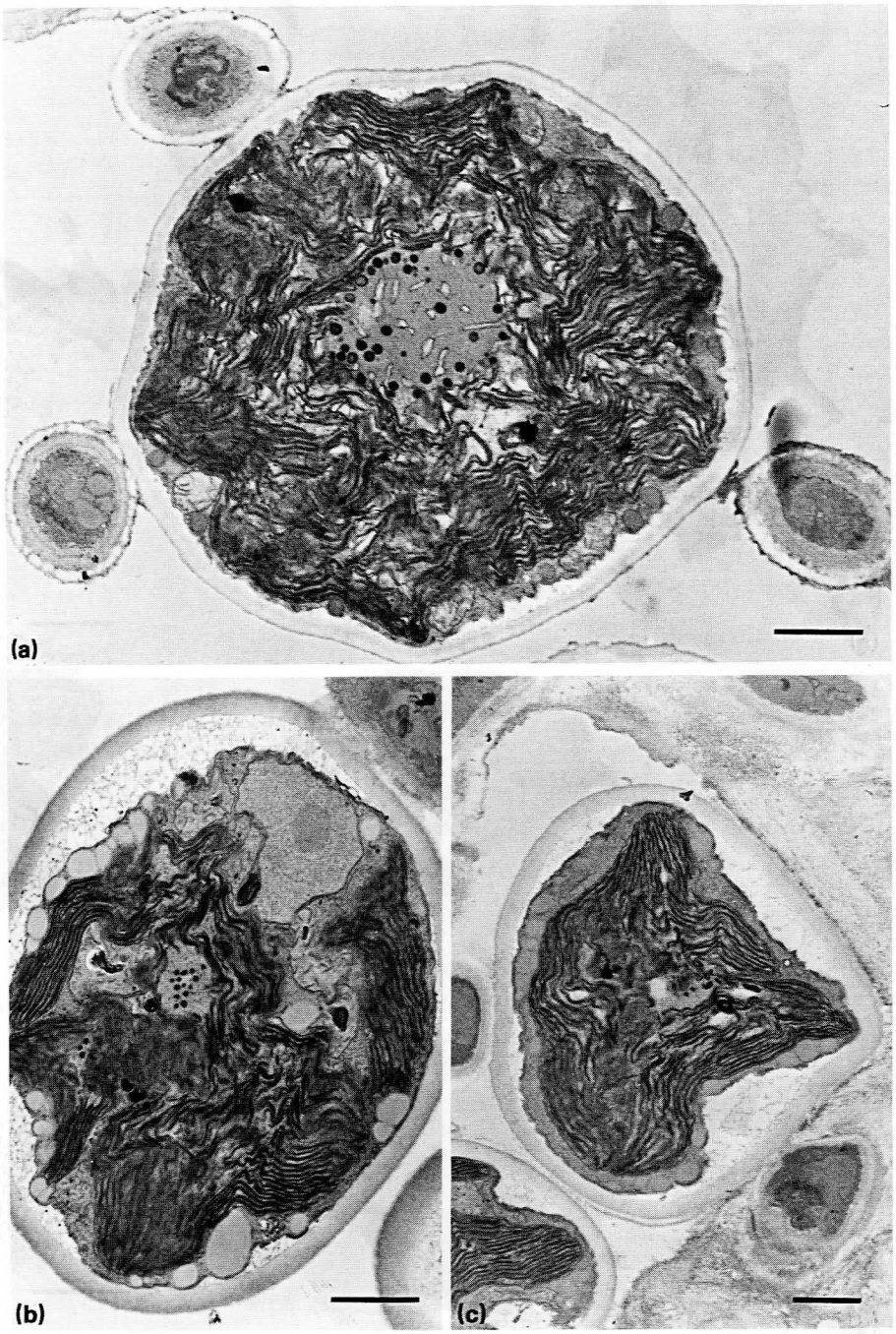
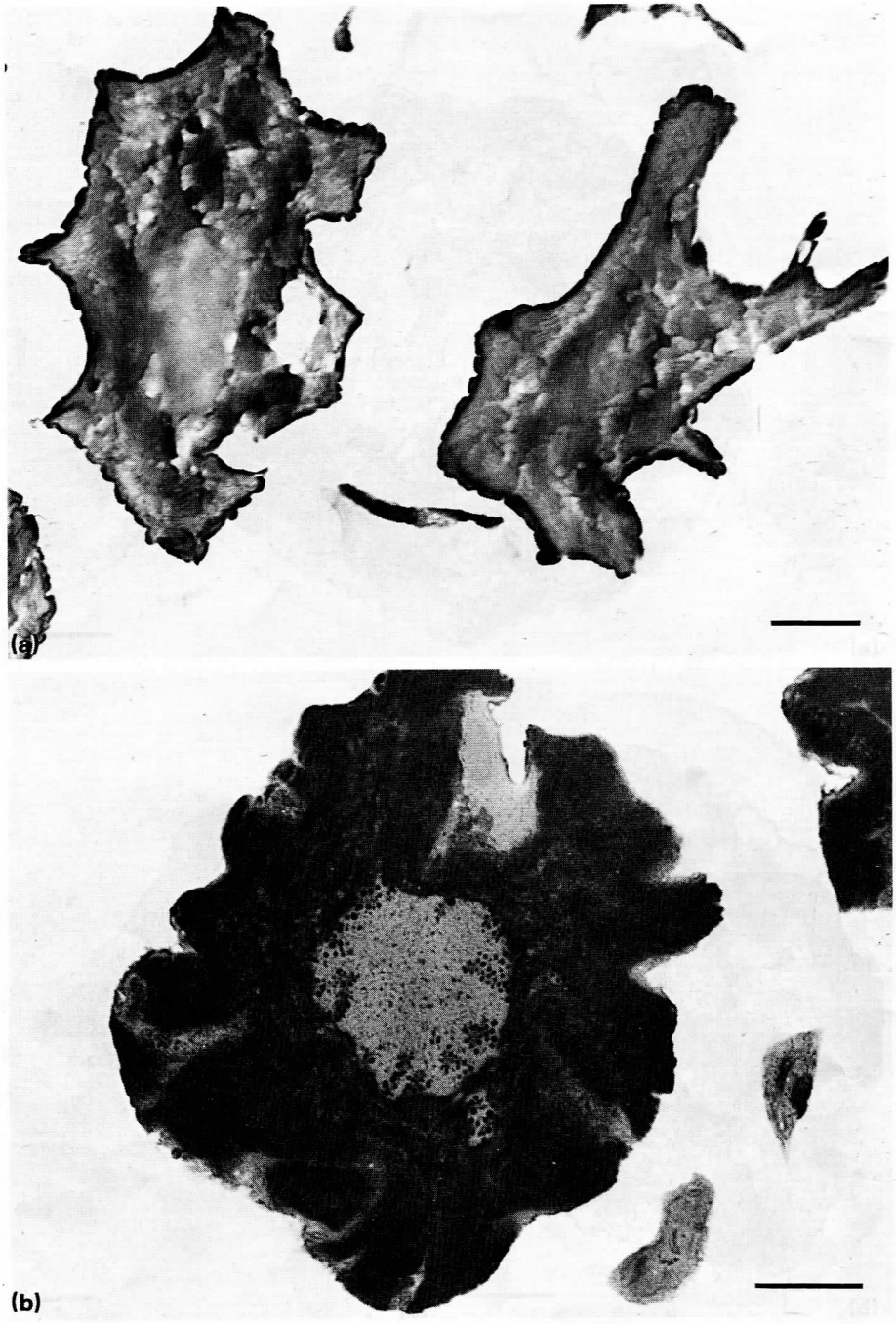


Fig. 1. For caption see page 299.



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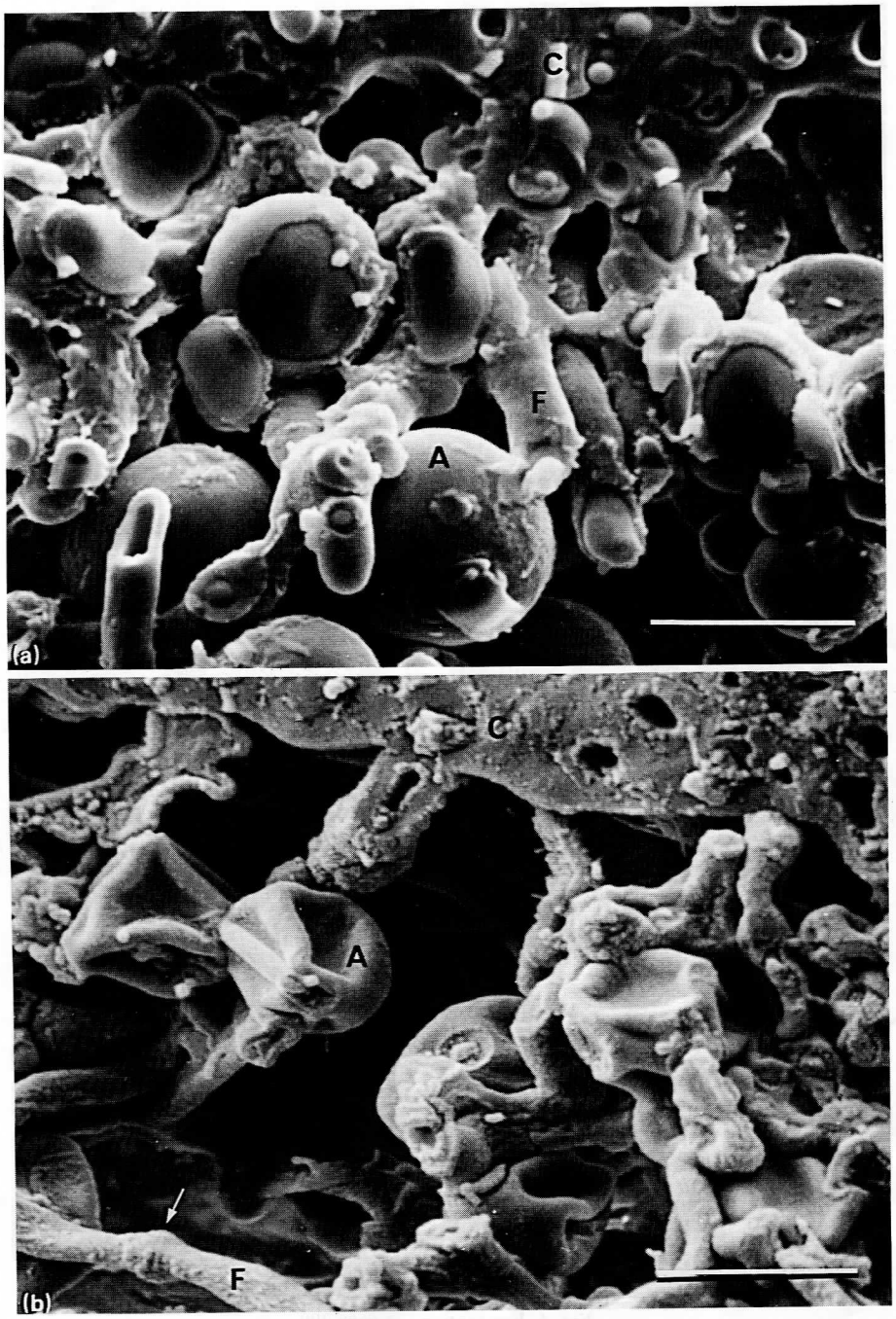

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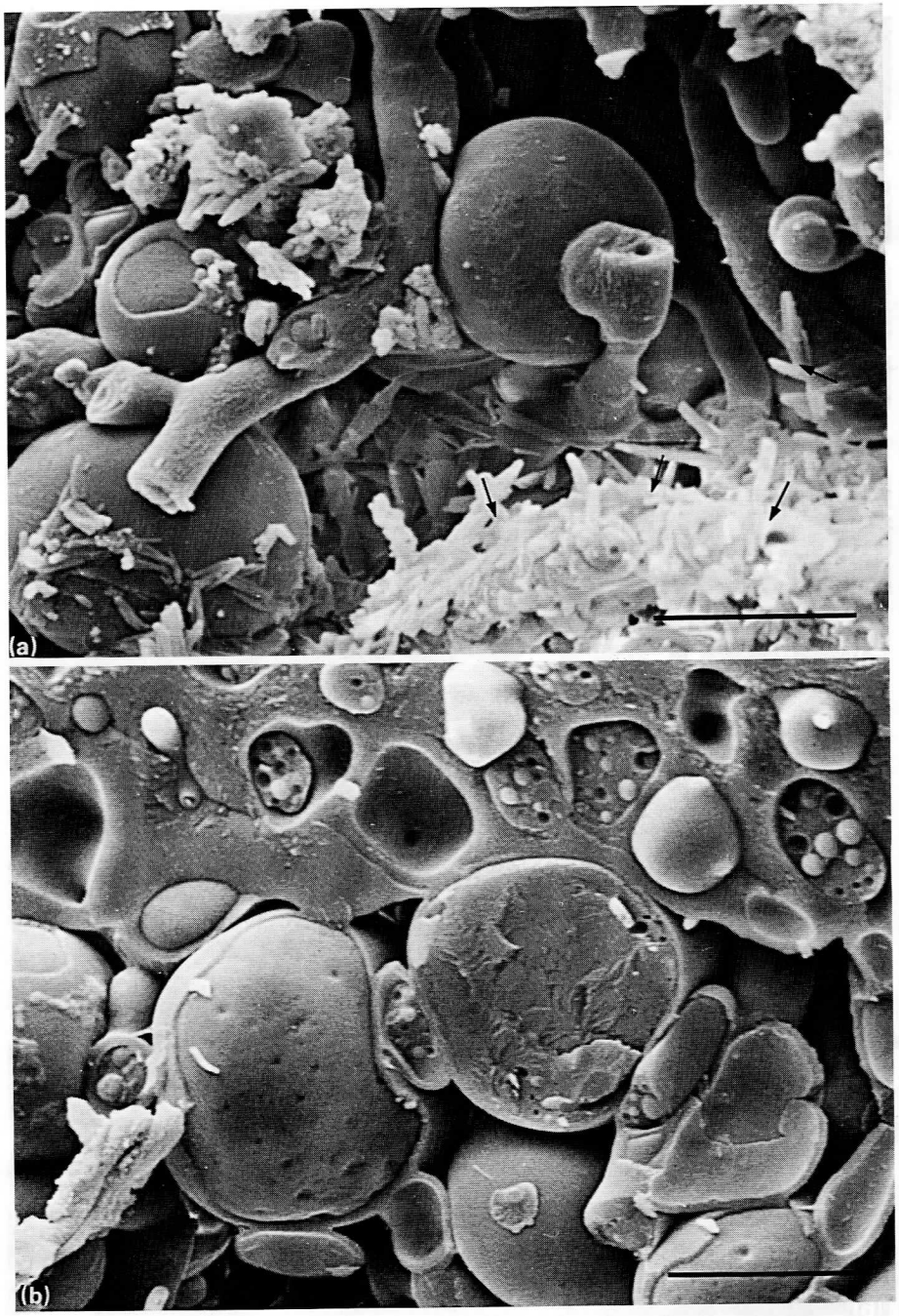

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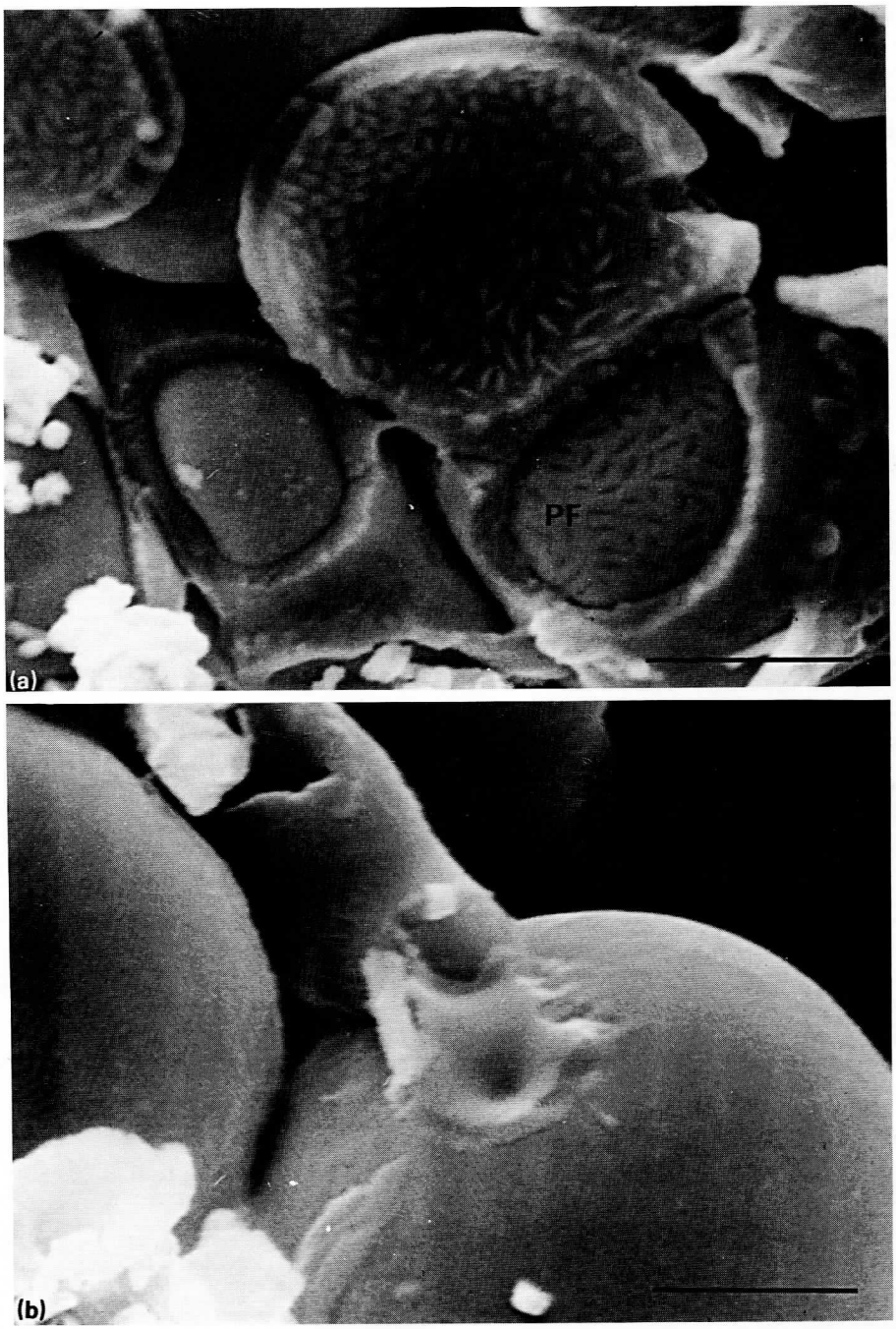

Fig. 5. For caption see page 299.

Explanations of Figures

Fig. 1. Sections of algal cells in *Parmelia sulcata* fixed in solution after storage at (a) 100% RH or (b) and (c) 0% RH. (a) and (b) are cells from within the algal layer and (c) is a cell adjacent to the cortex. Bar = 1·0 μm.

Fig. 2. Sections of algal cells in *Parmelia sulcata* fixed in osmium tetroxide vapour after storage at (a) 0% RH or (b) 100% RH. Bar = 1·0 μm.

Fig. 3. Parts of freeze-fractured, frozen-hydrated thalli of *Parmelia sulcata* after storage at (a) 100% RH or (b) 0% RH. A, algal cell; F, fungal hypha; C, cortex. Arrow indicates septum in hypha. Bar = 10 μm.

Fig. 4. Parts of freeze-fractured, frozen-hydrated thalli of *Parmelia sulcata* rehydrated for 2 min with (a) water or (b) glutaraldehyde-phosphate buffer after storage at 0% RH. Arrows indicate crystals on hyphae. Bar = 10 μm.

Fig. 5. Parts of freeze-fractured, frozen-hydrated thalli of *Parmelia sulcata* stored at 100% RH, showing (a) ridges on the plasma membrane and (b) interior of a fungal hypha in contact with an algal cell. EF and PF, exoplasmic and protoplasmic plasma membrane faces. Bar = 1·0 μm.